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THE APPLICATION OF STANDARD SI UNITS AND TERMODYNAMICS IN DETERMINATION OF HERBICIDES' INFLUENCE TO MAIZE INBREDS
1. The growth.

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From 15 maize inbreds, submitted to alachlor and atrazine treatment in controlled conditions, after 14 days growing in sand under distilled water, fresh and dry weight and length of root and shoot were determined. The parameters derived of those measurements: concentration (gL^{-1}), root:shoot relation and pseudospecific density (*d,* d^{-1}, $\mu mol\ mg^{-1}$) classified the mechanisms of herbicide influence in whole plants and roots or shoots. Thus, alachlor lowered weights, lengths, root:shoot relation and elevated concentration in all inbreds, underlining the suppression of water input and root as the target. The atrazine, on the contrary, downed concentration, promoting the water-induced growth. The applications of diluted or combined herbicides' forms segregated the genotypes to tolerant or sensitive as whole plant or partially root or shoot,

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only. The pseudo-specific density separated the genotypes to non- or stable system to hold up the induced change.

Key words: alachlor, atrazine, maize inbreds, pseudo-specific density, root to shoot relation, tissue concentration

INTRODUCTION

Inbred lines, as monosigots maize plants, are susceptible to weed presence (STEFANOVIĆ, 1992; STEFANOVIĆ and BUDIMIR, 1998) and to herbicides' activity (BREAUX *et al,* 1987; JAWORSKI *et al*, 1975). Those facts have to be taken in a count in maize seed production, what makes this process more complex. In maize seed production practice, herbicides are usually used for weed control. So, it is necessary to examine reaction of inbreds to herbicides to find the best solution in weed control, without damage of maize inbreds at the same time, with present differences in sensitivity to herbicides between genotypes.

The absorbed herbicide set up quick side reactions' cycles in maize plants, with some protective molecules, like glutathione (JAWORSKI *et al*, 1975), what disorders the main reactions of biosynthesis in run. During the process of selection it is important to give estimation about resistance of particular inbred to particular herbicide. Hence, the selection operates with a huge number of qualitative units, while it is necessary to select highly sensitive, quick and chip exact testing methods. The combination of biological, chemical and physical parameters, like root to shoot relation, concentration and pseudo specific density were shown to be very useful (effective) in expressing of maize and soybean's replay to stress caused by pesticides and low temperature (SREDOJEVIĆ *et al*, 2005 a, b).

In this essay those parameters were applied to the trial with 15 maize inbreds of older generation treated by two herbicides and their combination to determine the reply of genotypes to treatments.

MATERIAL AND METHODS

The essay with 15 maize older generation inbreds, which were subjected to preemergence treatment with herbicides: alachlor (AL) 10^{-4} M and 10^{-5} M (2-hlor-2',6'-dietil-N-metoksimetilacetanilid), atrazine (A) 10^{-4} (6-hlor-N2-etil-N4-iyorpoil-1,3,5-triayin-2,4-diamin) and their combination in ratio 2; L+A 10^{-4}M was investigated in controlled conditions of phytotron. Those conditions mean: day/night temperature of 25^{o}C/18^{o}C and photoperiod of 12 h, relative humidity of 75 % and illumination of 1250 lux. The planting (3x50 seeds) was done in germinating chamber with sand and 10% of solutions; the humidity was kept by distilled water. After 2 weeks (fool growth of three leaves) there was counted a sprouting in each chamber, then, length and measured weight: fresh and dry (60^{o}C; until constant weight) of root and shoot. At the same conditions the control, i.e. non-treatment was done, too. All collected parameters were expressed per plant or plant sequence (root and shoot) and statistically analysed (STEFANOVIĆ *et al* 1990; STEFANOVIĆ and BUDIMIR, 1992).

Gravimetrically determined fresh (FW) and dry (DW) weights were used to calculate water content as volume (Vw), while g = ml:

$$Vw\ (cW) = FW - DW \qquad [1]$$

The two forms of weights (F and D) and water content was used to calculate the concentration of dry mater (conc, g L^{-1}) and pseudo specific density (d^{-1}, *d*, μmol H_2O mg $^{-1}$ FW).

The root to shoot lengths' relation (rt:sh) was used in consideration of growth's stability.

RESULTS AND DISCUSSION

In the all examined inbreds, submitted to the treatment of two herbicides, there were detected the changes in growth, i.e. fresh and dry weight, then, the length of root and shoot (Table 1; 2; 3) as it was discussed in previous papers (STEFANOVIC, 1992; STEFANOVIC and BUDIMIR, 1998). Meanwhile, the application of some inner biological, chemical and physical parameters, calculated from those basically measurements could help in separation of types of changes, depending on herbicide and the reply of genotype, by the whole plant, root or shoot.

Tab. 1. - The yield of fresh weight of non-treated (control) and treated seedlings by herbicides (mg plant^{-1})

	Shoot					Root				
Genotype	Contol	AL 10^{-5} M	AL 10^{-4} M	A 10^{-4} M	AL+A 10^{-4} M	Contol	AL 10^{-5} M	AL 10^{-4} M	A 10^{-4} M	AL+A 10^{-4} M
L1	1395	525	471	719	1105	502	440	167	724	690
L2	874	596	435	697	660	1109	773	409	818	801
L3	557	518	499	611	483	954	865	763	948	707
L4	578	425	205	510	634	706	525	386	816	634
L5	575	349	140	331	379	584	467	173	476	418
L6	537	531	282	620	517	640	583	294	696	455
L7	674	800	123	658	613	374	719	177	934	525
L8	478	405	388	537	596	654	491	459	645	589
L9	414	467	294	453	426	637	539	396	610	490
L10	526	361	285	631	609	476	407	261	839	659
L11	395	479	360	546	449	561	573	479	734	537
L12	329	361	283	530	406	460	360	285	491	434
L13	540	377	441	639	510	547	370	435	723	529
L14	290	409	235	521	426	417	450	237	519	502
L15	409	430	269	543	600	387	419	356	761	586

Control - non-treatment; AL - Alachlor; A- Atrazine; AL+A - Alachlor + Atrazine

The relation of root to shoot length and the concentration (g L^{-1}) were parameters with highest oscillations in this essay. Hence, the r:sh relation is measure of root and shoot growth's balance; while, the dry substance concentration in water connects the changes in water accumulation and new-substance synthesis. Thus, in alachlor 10^{-4}M treatment the shortening of r:sh relation was caused by reduction of root's size (compared to shoot size) exactly in all examined inbreds at least L' (L5). Meanwhile, the shoot's length was shortened at the same time; meaning that root was more sensitive target of herbicide action (Tab. 3). Despite of both size reductions, the dry substance concentration in root and shoot was enlarged even 5 times in L4 and L7 shoots, while it was diminished in L8 root (92%), then, in alachlor's treatment (AL 10^{-5}M) reduced the concentration in root and shoot of L6 (71% and 88%) and L15 (66% and 44%); then, it was alternated in root or shoot over and below non-treated like in L4, L9, L12, L13 and L14, and elevated in L1, L5, L10, L12 (Fig. 1). The applied agent alachlor was disordered the water input in both dilutions (10^{-4} and 10^{-5}M), the shortened root and shoot, then, diminished their fresh and dry weights (Tab. 1, 2 and 3) and nearby of these elevated the concentration, what pointed out that the main disorder was in intake and distribution of water (Steudel, 2000; Hsiao and Xu, 2000). The responsibility lies in root, which was retained inputted water to dilute own system (Prichard *et al*, 2000), then, it is connected to the extremely elevated concentration in shoots of L4 and L9 (5 times higher then non-treated), (Fig.1).

Tab. 2 - The yield of dry weight of non-treated (control) and treated seedlings by herbicides (mg plant^{-1})

	Shoot					Root				
Genotype	Contol	AL 10^{-5} M	AL 10^{-4} M	A 10^{-4} M	AL+A 10^{-4} M	Contol	AL 10^{-5} M	AL 10^{-4} M	A 10^{-4} M	AL+A 10^{-4} M
L1	188	122	109	118	165	94	134	57	117	124
L2	158	97	103	97	108	236	125	111	121	144
L3	88	96	115	109	111	130	164	149	167	146
L4	116	121	115	97	156	160	133	149	132	186
L5	115	112	51	69	83	104	139	65	88	96
L6	102	89	98	100	117	157	103	84	106	125
L7	116	133	64	105	130	87	142	74	146	119
L8	143	120	88	100	129	150	114	99	144	140
L9	106	126	79	107	106	137	147	96	134	135
L10	99	103	84	120	131	92	127	85	152	147
L11	72	92	79	97	109	108	109	105	145	148
L12	95	143	105	138	104	115	148	111	192	122
L13	271	99	248	108	132	253	108	238	141	169
L14	88	93	66	97	106	99	115	84	100	138
L15	158	93	128	102	162	150	124	140	147	193

Control - non-treatment; AL - Alachlor; A- Atrazine; AL+A - Alachlor + Atrazine

Tab. 3 - The length of shoot and root (cm) and root:shoot ratio

Genotype	Shoot					Root					Root.shoot ratio				
	Contol	AL 10^{-5} M	AL 10^{-4} M	A 10^{-4} M	AL+A 10^{-4} M	Contol	AL 10^{-5} M	AL 10^{-4} M	A 10^{-4} M	AL+A 10^{-4} M	Contol	AL 10^{-5} M	AL 10^{-4} M	A 10^{-4} M	AL+A 10^{-4} M
L1	12	-	6,36	9,9	4,32	12,01	-	11,5	11,61	10,34	1,00	-	2,39	1,81	1,17
L2	17,33	-	9,84	19,55	8,44	14,48	-	14,18	14,87	11,83	0,84	-	1,40	1,44	0,76
L3	19,48	-	5,84	16,24	12,34	11,04	-	10,13	11,77	10	0,57	-	0,81	1,73	0,72
L4	16,66	-	8,64	17,58	9,82	12,34	-	9,62	12,51	10,44	0,74	-	1,06	1,11	0,71
L5	14,48	-	5,65	11,98	7,12	12,47	-	6,58	11,12	8,28	0,86	-	1,16	1,16	0,93
L6	18,86	-	5,89	17,33	8,85	13,83	-	11,99	13,49	11,65	0,73	-	1,32	2,04	0,78
L7	13,94	-	6,59	18,76	6,9	13,03	-	8,79	13,44	11,63	0,93	-	1,69	1,33	0,72
L8	10,25	-	6,52	12,14	5,16	12,46	-	11,48	13,24	9,16	1,22	-	1,78	1,76	1,09
L9	16,54	-	6,4	17,33	7,09	12,56	-	9,54	14,15	9,39	0,76	-	1,32	1,49	0,82
L10	13,02	-	4,43	12,02	6,15	11,05	-	10,96	10,52	9,27	0,85	-	1,51	2,47	0,88
L11	19,2	-	7,3	18,6	8,17	16,88	-	12,44	15,03	12,99	0,88	-	1,59	1,70	0,81
L12	10,65	-	5,3	10,39	5,76	14,43	-	10,03	14,32	13,46	1,35	-	2,34	1,89	1,38
L13	10,05	-	6,04	14,88	5,39	10,17	-	10,71	13,85	8,12	1,01	-	1,51	1,77	0,93
L14	13,27	-	6,29	13,2	7,2	10,58	-	11,53	11,58	9,6	0,80	-	1,33	1,83	0,88
L15	16,31	-	5,09	17,36	5,59	12,74	-	9,66	14,15	9,49	0,78	-	1,70	1,90	0,82

Control - non-treatment; AL - Alachlor; A- Atrazine; AL+A - Alachlor + Atrazine

The basic characteristic reaction to atrazine application was reduced concentration in the majority of inbred lines, with extremely low concentration in L13 (28% in root and 20% in shoot) and L15 (38% in root and 37% in shoot). Although, the values of concentration over non-treatment, were observed in L3 (16% and 35% in root and shoot, respectively) and L12, in root (2 times over non-treatment); there were inbreds, which hold the concentration closely to non-treatment (L5, L9, L10 and L11 in root and shoot). The next characteristic of this agent was the hold on or slight fluctuation of rt:sh relation (78% to 112%), with exception of rise (34%) present at L7 and decrease (22%) present in L3 (Tab.3). Meanwhile, in the combined treatment, the root was shortened, as well as, the concentration was elevated moderately in L3 root (65%) and about 50% in root and shoot of L3, L4 and L11, supposed by the synergy effect of two herbicides (AL+A 10^{-4}M, Fig.1), (VARSANO *et al,* 1992). The inbreds L13 and L15 retained the low concentration in root (55% and 78%) and shoot (35% and 59%), expressing their susceptibility to atrazine, as it was shown in previous discussion of pure atrazine.

Hence, very low concentration in root (L2, L6 and L7), shoot (L5 and L8) and both (L13, L14 and L15) pointed out the occurrence of unfavourable, water induced growth (BOYER, 2001; TANG and BOYER, 2001). In general, the applied herbicides and their combination influenced the preeminence of examined genotypes to susceptible and tolerant, according to changes in whole plant and partially, in root or shoot. Such segregation enables the choice of herbicide species (depended on reactive centres and mechanisms of reactions) for application in determined inbreds' crop.

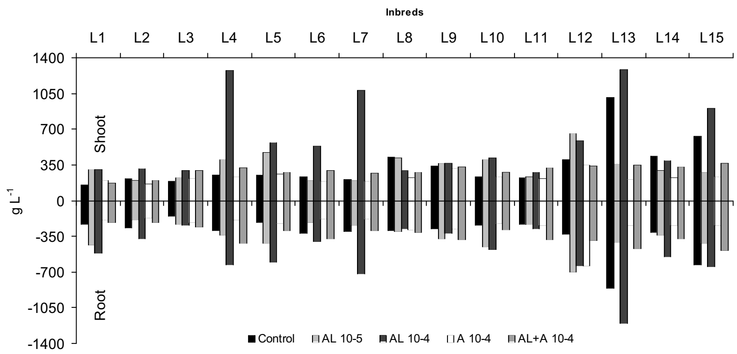

Fig. 1. - The concentration of dry substance in shoot and root;
Control - non-treatment; AL - Alachlor; A- Atrazine; AL+A - Alachlor + Atrazine

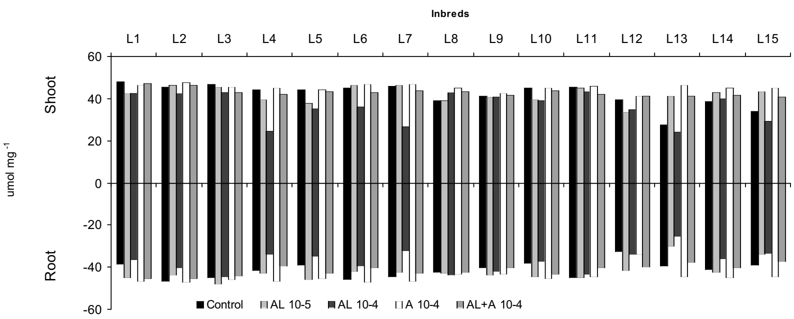

Fig. 2. -Pseudo-specific density of shoot and root;
Control - non-treatment; AL - Alachlor; A- Atrazine; AL+A - Alachlor + Atrazine

The pseudo-specific density, *d*, as stable factor, that supported plant's system (root and shoot), was not changed in L2, L3, L9 and L11, on the contrary to L4, L5, L10 and L12, where *d* values downed in both dilutions of alachlor (10^{-4}M and 10^{-5}M). The other inbreds characterise the stable *d* values only in root or shoot, independently of agent's dilution. Meanwhile, in atrazine treatment and its combination with alachlor, only in L4 L12, and L13 inbreds *d* values of root were lowered. The majority of genotypes had the *d* values in root and shoot over 40 μmol mg^{-1} and below 50 Symbol μmol mg^{-1}, then, a few between 30 μmol mg^{-1} and 40 μmol mg^{-1} and only L4, L7, and L15 in shoot and L13 in root and shoot below 30 μmol mg^{-1}. The essays with low temperature application to four soybean genotypes in three types of soils, then, nine maize genotypes treated with five pesticides and one soybean treated with two lower dose of cadmium had *d* values between 40 μmol mg^{-1} and 50 μmol mg^{-1}. Only in over dose of cadmium (3 ppm treatment) *d* value was below 40 μmol mg^{-1} (SREDOJEVIC *et al* 2005b).

The segregation of genotypes to resistant and susceptible root and shoot (and vice versa) could be attributed (addressed) to mechanisms of absorbed herbicide's fragmentation to inactive molecules. Namely, alachlor inactivation goes by quick reactions upon glutathione, which in examined maize inbreds leaved hard disorder in length, then, fresh and dry weight, which were in lesser extent expressed with dilution (AL 10^{-5}M and herbicides' combination 2:1 10^{-4}M). This is already known that atrazine has separate mechanism of its deactivation, i.e. non-enzymatic in root and through glutathione cycles in shoot (BREAUX *et al,* 1987; JAWORSKI *et al,* 1975). Further, the genetic basis of genotypes' differentiation at susceptibility could come from the degree of completing the other proteins in crop with absorbed herbicides (MANCH and DUDLER, 1993). It is well known that the proteins are water-binding centres forming free, chemically and structurally incorporated water (KRISHNAN *et al,* 2003; SREDOJEVIC *et al,* 2005a).

In conclusion: According to the trend of changes in fresh and dry weights in this essay, the main disorder was moved up from water content and its distribution. They could be generated as causal-primary, and/or consequence-secondary, by changes of mechanisms with herbicide input, but the range - what was the first and what the second, had no meaning after 14 days of plants' growth. From this point of view, the resistant genotypes to the treatment of herbicides could be accepted these, which in whole (plant) or partially (root, shoot) experienced lowest difference to non-treatment in: concentration, the connecting factor of the substance in reaction and the medium of reaction; then, pseudo-specific density, the factor which mediates total plant's mass (fresh weight) to water mass as hugest part of this, and, as the consequence, to hold on the balance of growth.

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UPOTREBA STANDARDNIH SI JEDINICA I TERMODINAMIKE PRI ODREĐIVANJU UTICAJA HERBICIDA NA SAMOOPLODNE LINIJE KUKURUZA
1. Rast.

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I z v o d

Klijanci 15 samooplodnih linija kukuruza bili su izloženi tretmanu alahlora i atrazina, u kontrolisanim uslovima klijališta (gajeni su 14 dana na peščanoj podlozi, uz zalivanje sa destilovanom vodom). Bila su izvršena merenja sveže i suve mase, dužine korena i izdanka. Dobijeni parametri su poslužili za izračunvanje: koncentracije (gL^{-1}), koren: izdanak relacije i pseudospecifične gustine (*d,* d^{-1}, $\mu mol\ mg^{-1}$). Navedene veličine su primenjene za definisanje uticaja herbicida, kako na cele klijance, tako i na njihove pojedine delove: koren i izdanak. Tako je alahlor uticao na smanjenje sveže i suve mase, dužine i relacije korena i izdanka, uz povećanje koncentracije, kod svih linija. Potrebno je naglasiti i smanjenu apsorpciju vode u koren, kao mesto sa najintenzivnijom reakcijom na alahlor. Sa druge strane, atrazin je smanjio koncentraciju, vodeći tzv. vodom indukovanom porastu. Tretmani sa većim razblaženjem ili kombinacijom herbicida definisali su linije kukuruza na tolerantne i osetljive, preko celih klijanca ili njihovih delova, tj. korena i izdanka. Pseudospecifična gustina je poslužila za determinisanje linija prema stabilnosti sistema da izdrži indukovanu romenu.

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